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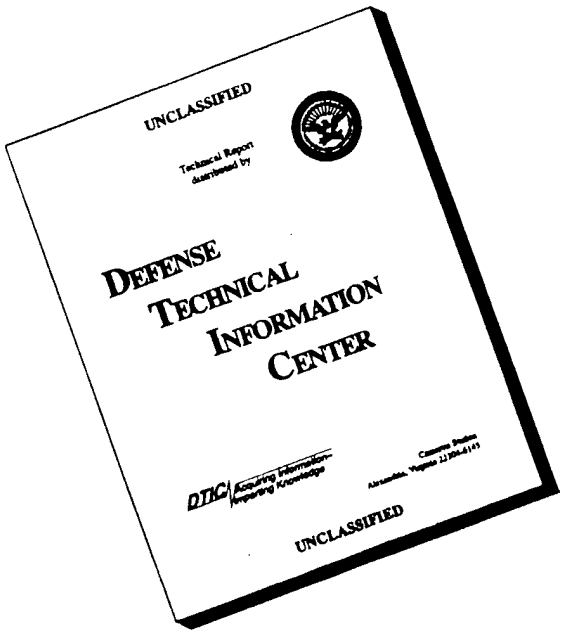

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Role of Counterions in the Gigahertz Relaxation of Wet DNA

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ABSTRACT

We have measured the dielectric properties of concentrated solutions and gels ($\leq$30 mg/ml) of random-sequenced DNA from E.coli in the 400 MHz to 26 GHz range. Two Debye-type relaxations are evident, one with a relaxation time near 9 ps and attributable to the classical Debye relaxation of water. More noteworthy is a second relaxation process with a characteristic time in the 20 to 200 ps range, i.e. a relaxation frequency in the 0.8 to 8 GHz range, depending upon the species of the counterions and the temperature. The slower relaxation process has an enthalpy of 3.3 kcal/mol and is accounted for by a counter-ion-based relaxation process. These experimental results are considered in terms of two models from polyelectrolyte theory, one by Oosawa and Wyllie and the other by Manning, and we propose that different ion-based relaxation mechanisms dominate in different hydration regimes.

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There is considerable experimental evidence (1-8) indicating the occurrence of far-infrared (<200 cm-1) vibrational modes in nucleic acids. These vibrational or phonon modes are characterized by in-phase motion extending over many nucleotides and it is believed that these modes mediate structural transitions and may serve as an avenue for energy transport. Any biological relevance crucially depends on the lifetimes of these modes. Theoretical models (9-12) give a general description of the low-frequency dynamics of nucleic acids; however, a detailed understanding of the damping mechanism(s) governing the solvent-biopolymer interface is lacking. Previous experimental investigations have, however, made progress in this regard. Coupled-mode theory has been implemented to account for Raman and Brillouin investigations of DNA gels as a function of temperature (3) and hydration (4) where the damping mechanism was attributed to a nucleic-acid-water relaxation. The coupled-mode theory produced relaxation times in the gigahertz frequency range, indicating that dielectric techniques may be used to directly probe the relaxation process(es). Here we report on our experimental investigation of the dielectric properties of DNA.

Vector dielectric measurements were carried out with a Hewlett Packard 8510 Network Analyzer where a coaxial line (13) is terminated at the surface of several milliliters of either calibration or sample solutions. Accurate determination of the permittivity of the solutions requires a calibration model (14) to remove the effect of internal reflections inherent to the apparatus. We have found that the optimum calibration procedure

for the open-ended-coaxial-line technique is based on the measurements of an open circuit and two known solutions with well characterized salinity and temperature (15); the dielectric properties of the two calibration solutions should bracket the dielectric properties of the sample. To analyze the permittivity data we have modified a commercial software package (LAB CALC, Galactic Industries) based on the Levenberg-Marquardt method (16,8) to fit the permittivity as a sum of Debye relaxation processes and a conductivity term (17):

$$\varepsilon' = \sum_i \frac{\Delta_i}{1 + \omega^2 \tau_i^2} + \varepsilon_\infty$$

$$\varepsilon'' = \sum_i \frac{\Delta_i \omega \tau_i}{1 + \omega^2 \tau_i^2} + \frac{\sigma}{\varepsilon_o \omega}$$

where ε' and ε'' are the real and imaginary parts of the permittivity, τ_i and Δ_i are the relaxation time and the dielectric increment of the i^{th} relaxation process, ε_∞ is the infinite frequency dielectric constant, σ is the conductivity, ε_o is the permittivity of free space and ω is the angular frequency. To demonstrate the capabilities of both the experimental technique and the data analysis, figure one presents both measurements of a known saline solution and the results of fitting the data with a single Debye relaxation process. The Debye model works remarkably well in this frequency regime.

NaDNA from calf thymus, with a wide distribution of lengths, was purchased from Sigma Chemical Company (lot 109F9540). The salting of the sample solutions was established in a multi-step process. First, the lyophilized NaDNA was dissolved in 100 mM NaCl, LiCl, KCl, CsCl, MgCl2, or CaCl2 solutions. Two-and-one-

half volumes of ethanol were added and the DNA was precipitated at -20 Celsius. The resulting pellet was redissolved in the appropriate solution and this process was repeated to complete the salt exchange. The final pellet was dried under vacuum and then placed in a solution ranging from 10 to 100 mM salt and with DNA concentrations ranging from 1 mg/ml to 30 mg/ml, resulting in solutions at the lower concentrations and concentrated gels at the higher concentrations.

Measurements were initially made in the 400 MHz to 26 GHz range. It soon became clear, however, that no new information was provided at the higher and relatively noisier frequencies and thus the experimental frequency range was limited to 400 MHz to 10 GHz, as displayed in figures one and two. Figure two presents measurements and curve fitting results for CaDNA at room temperature: this data is optimally fit with two Debye relaxations. Table one summarizes the results of the CaDNA measurement and of similar measurements of CsDNA, KDNA, LiDNA, MgDNA, and NaDNA, which uniformly require two Debye relaxation processes to fit these data. In general terms, these measurements of concentrated DNA gels exhibit a dominant relaxation process with a relaxation time near 9 ps, characteristic of bulk water (18), and a less prominent relaxation process with a relaxation time in the 20 to 200 ps range depending on the species of counterions and the temperature. This range of relaxation times corresponds to a relaxation frequency range of 0.8 to 8 GHz. Figure three

presents an Arrhenius plot of KDNA yielding an enthalpy of 3.3 kcal/mol.

The most remarkable feature of these data is the occurrence of a counterion-dependent relaxation process with a relaxation time in the 20 to 200 ps range. With regards to this gigahertz relaxation process, the LiDNA and MgDNA data are fit with the shortest relaxation times, CsDNA and KDNA data the longest, and the CaDNA and NaDNA data exhibit intermediate relaxation times (see table one).

We have considered these experimental results in terms of both the theory of dielectrics and polyelectrolyte theory. Frohlich (19) has shown that the Debye equations follow from an exponential decay function and has considered several models for which these equations hold. For an ion-based mechanism the relevant model is essentially a double-well potential where the height of the potential barrier is given by the enthalpy. Dielectric theory, however, does not provide a detailed description of the interaction of the ions with their local environment. There are two models from polyelectrolyte theory that address the local environment and are particularly relevant to these results. In an earlier theory, Oosawa (20) and Wyllie (21) modelled the dielectric dispersion in linear polyelectrolytes by considering the thermal fluctuations of bound counterions. The polyion was modelled as a uniformly charged rod, the counterions were modelled as a dilute solution, and the motion was confined to one dimension. The theory relates the

relaxation time τ to the fluctuation length b, the counterion mobility u, and the temperature T:

$$\tau = b^2/(\pi^2 ukT)$$

where k is Boltzmann's constant. Oro and Grigera (22) have observed a 1 ns relaxation process corresponding to a fluctuation length of 11 angstroms and attribute this feature to "counterion fluctuations on short sections, probably in a direction transverse to the macromolecular axis." Within the counterion-fluctuation model, the 20-200 ps relaxation times observed here correspond to fluctuation lengths in the 1 to 5 angstrom range. It is somewhat intriguing that such a continuum theory yields a length scale suggestive of a multiwell potential with minima centered on the phosphate groups.

Alternatively, this data may be considered in light of Manning condensation theory (23,24). This theory distinguishes between counterions in direct contact with phosphate groups, termed "site bound" ions, and counterions, termed "territorial bound" ions, which form a Debye screening atmosphere in a delocalized cloud near the polyelectrolyte. Manning cites extensive experimental evidence that DNA in solution is dominated by territorial bound ions and proposes that the counterions condense in a concentrated cloud of counterions forming a thin cylindrical shell about DNA. Manning calculates that the shell has a counterion concentration of 1.2 M and the outer surface of the shell is 17 angstroms from the symmetry axis. Futhermore, DNA is stabilized by the formation of this condensed cloud and the enthalpy change

associated with an ion moving between free solvent and the condensed cloud is calculated to be 3.2 kcal/mol, in favorable agreement with the experimental value of 3.3 kcal/mol. These considerations suggest a relaxation process pictured as a double well potential where one well characterizes free ions and the other condensed ions. This dielectric relaxation is spatially localized about the outer surface of Manning's cylindrical shell.

As pointed out in the introductory paragraph, coupled-mode analyses of Raman and Brillouin measurements (3,4) indicate the occurrence of gigahertz relaxations in DNA films at known relative humidities. The dielectric relaxations have been attributed to a nucleic-acid-water relaxation, where the two observed relaxation times near 40 and 2 picoseconds correlate to the primary and secondary hydration shells, respectively. Can these relaxations be attributed to a nucleic-acid-ion mechanism? Light scattering measurements determine an enthalpy of about 5 kcal/mol which compares favorably with previous measurements of the enthalpy of ATP solutions (25). ATP serves as a model system for nucleic acids and the enthalpy of the ATP solutions has been accounted for in terms of a phosphate-ion interaction as summarized by Phillips (26). Such an ion-based mechanism may serve as an alternative explanation of the relaxation process indicated by the coupled-mode-based model of the light scattering data.

The experimental results and theoretical considerations discussed above suggest the possibility that different relaxation

mechanisms dominate in different hydration regimes. We propose that at high water content a condensed cloud forms and the dielectric relaxation is dominated by ions near the outer surface of the cloud while at lower water content the ions associate more intimately with DNA and the dielectric relaxation is dominated by a phosphate-counterion-based mechanism.

In conclusion, we have observed two Debye-type relaxations in dielectric measurements of concentrated DNA gels in the 400 MHz to 26 GHz range. Of most interest is the low-frequency process with relaxation times in the 20 to 200 ps range, time scales accessible to computational models of DNA-solvent interactions. This process is dependent on counterion species and concentration, indicating that a counterion-dependent relaxation process occurs at gigahertz frequencies. Comparison with other experimental results suggest the occurrence of two relaxation mechanisms, one due to site-bound ions and dominating the low-hydration regime and the other involving territorial-bound ions and dominating the highly hydrated regime. These results highlight the role of counterions in the damping mechanisms governing the DNA-solvent interface.

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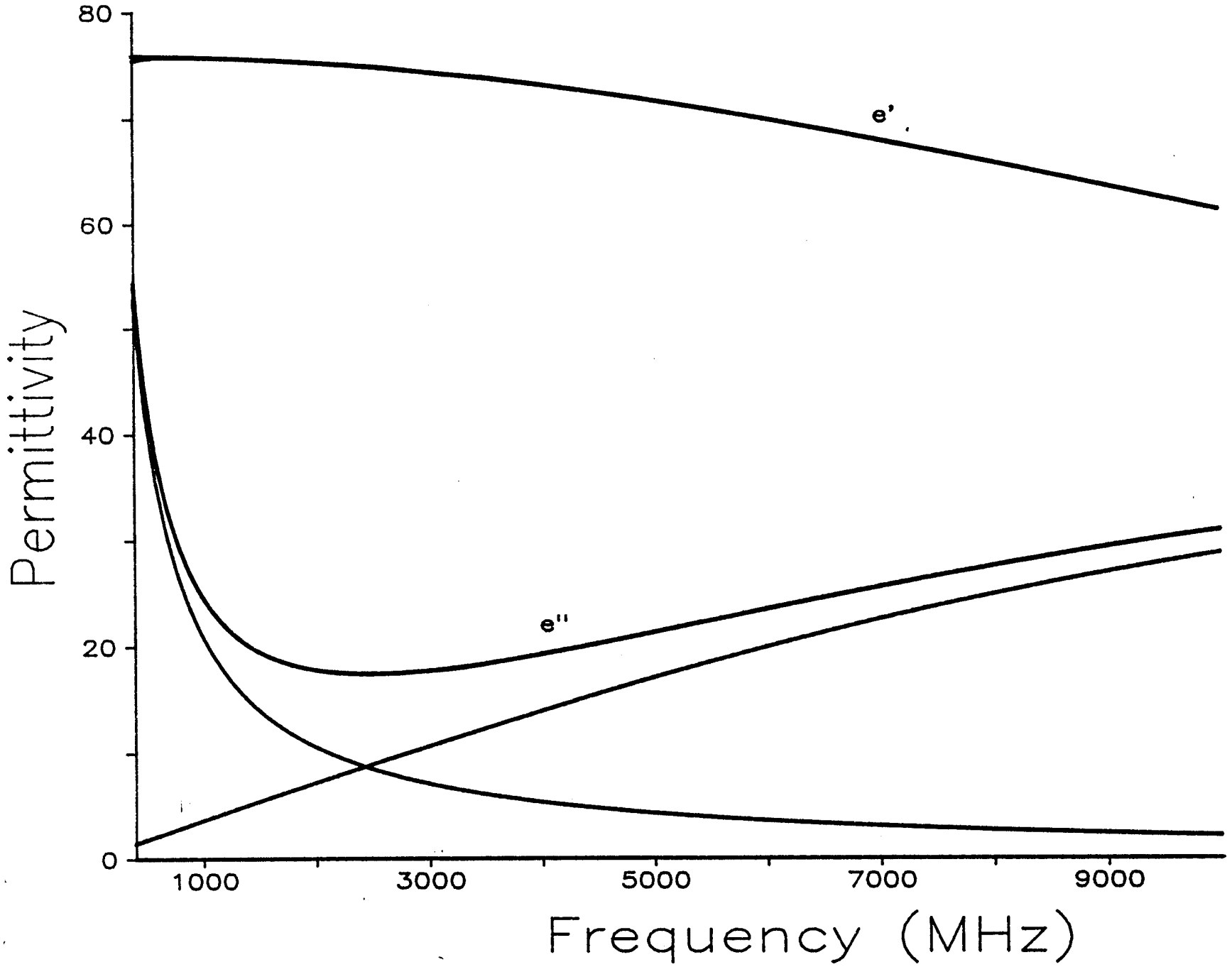
TABLE I. Relaxation times for concentrated DNA gels (near 25 mg/ml) at room temperature as determined by the nonlinear least-squares fitting of the permittivity data (see text).

Nucleic Acid	τ_w (ps)	τ (ps)
CaDNA	8.9	117
CsDNA	9.0	151
KDNA	8.9	170
LiDNA	9.2	103
MgDNA	8.9	96
NaDNA	8.6	134

Figure 1. Permittivity measurements of and curvefitting results for 100 mM NaCl at room temperature. The real (ε') and imaginary (ε'') parts of the permittivity as measured by the vector network analyzer are presented. These data are fit with a single Debye relaxation process and a conductivity term. For ε' the data and fitted curves are both plotted and are nearly coincident, exhibiting a minor mismatch at the lowest frequencies. For ε'' both the Debye relaxation of water and the conductivity curves are shown, and the sum of the two (plotted) is coincident with the data curve (also plotted).

Figure 2. Measurement of the imaginary part of the permittivity and curvefitting results for 25 g/l CaDNA at room temperature. The conductivity, Debye relaxation of water, and DNA-counterion relaxation are shown, and the sum of the three (plotted) is nearly coincident with the data curve (plotted).

Figure 3. Arrhenius plot of 30 g/l KDNA.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60
40
20
0
Permittivity
e'
e''
1000
3000
5000
7000
9000
Frequency (MHz)

Figure One

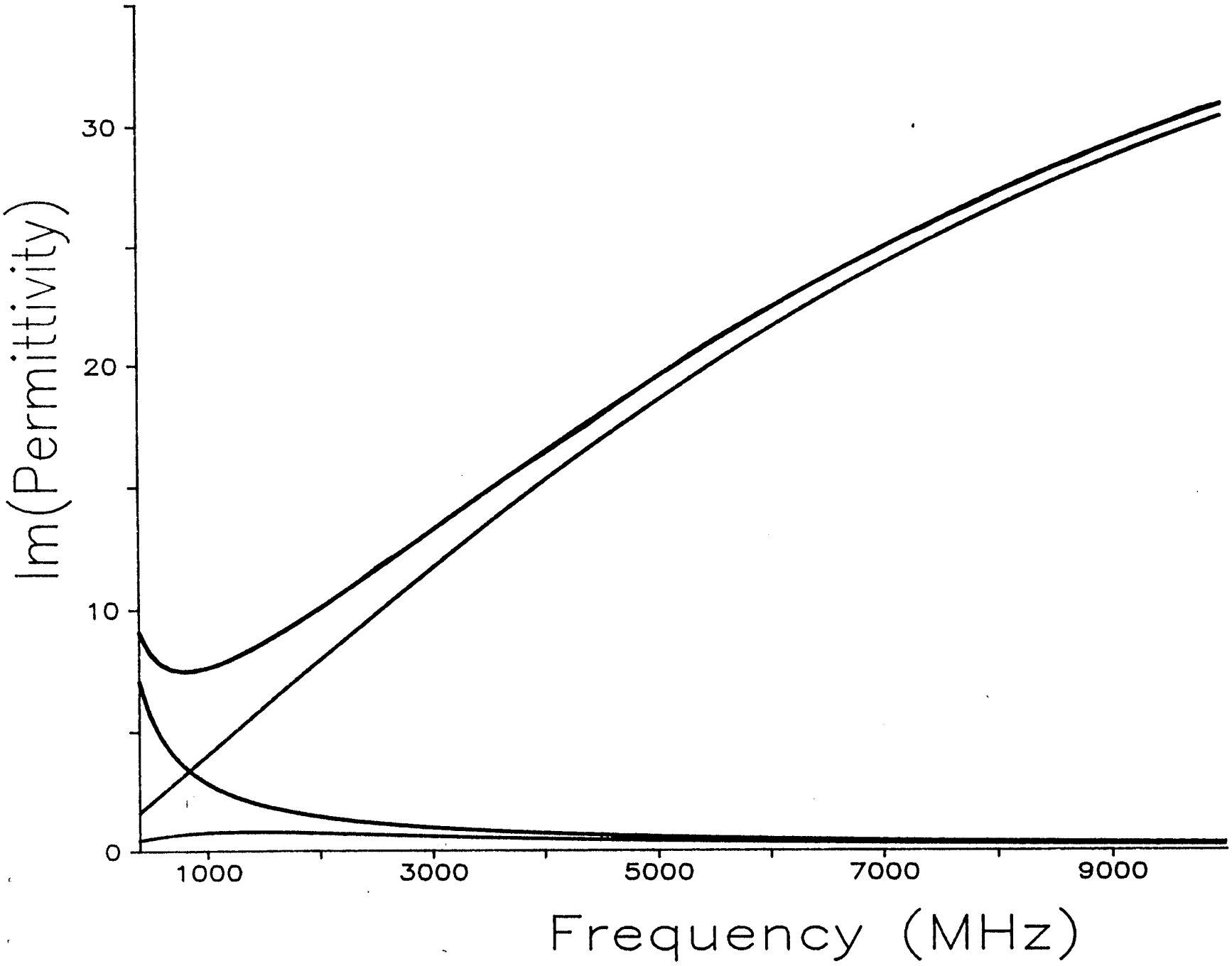
Im(Permittivity)
30
20
10
0
1000
3000
5000
7000
9000
Frequency (MHz)

Figure Two

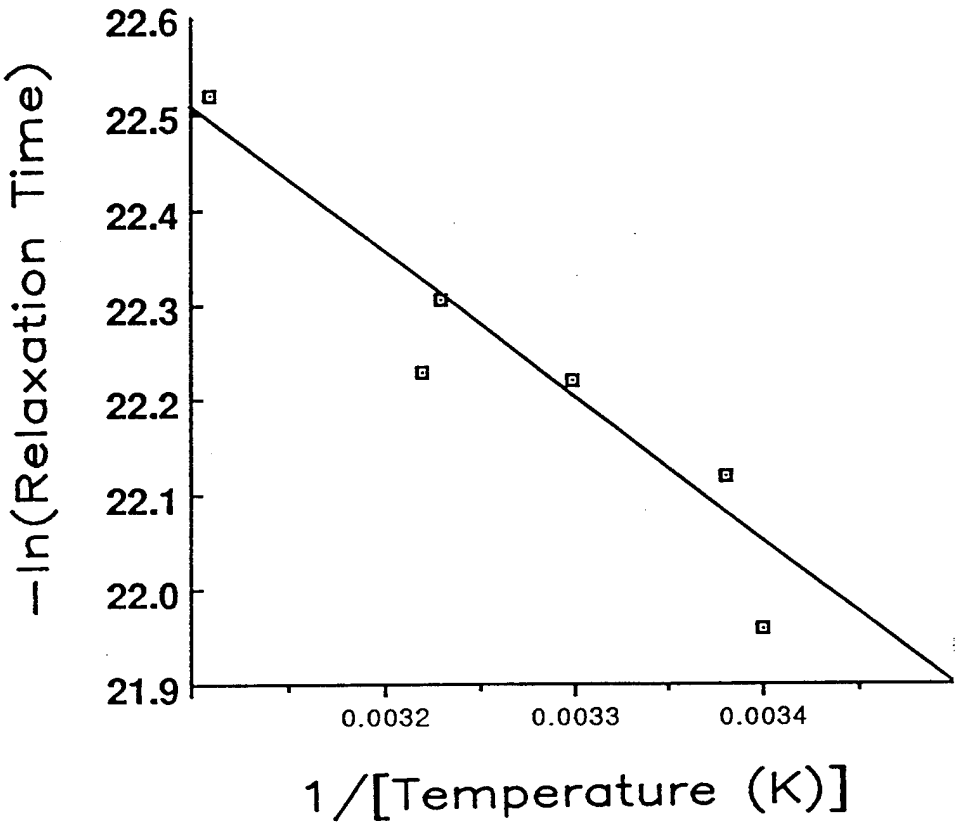

Figure Three